Application No. 09/785,793 Amendment dated September 29, 2003 Reply to Official Action of July 29, 2003



Amendments to the Claims:

This listing of claims will replace all prior versions and listings of the claims in the application.

Listing of Claims:

- 1. (currently amended) A Mmethod for detecting and/or purifying substances selected from proteins, biomolecules, and/or protein complexes, of proteins or biomolecules, subunits of biomolecule complexes, cell components, cell organelles and cells the method comprising the steps,
- (a) providing an expression environment containing one or more heterologous nucleic acids encoding one or more polypeptides and/or one or more subunits of a biomolecule complex, the polypeptides or subunits being fused to at least two different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A;
- (b) maintaining the expression environment under conditions that facilitate expression of the one or more polypeptides or subunits in a native form as fusion proteins with the affinity tags, and under conditions that allow the formation of a complex between the one or more subunits and other components capable of complexing with the one or more subunits;
- (c) detecting and/or purifying the one or more polypeptides or subunits by a combination of at least two different affinity purification steps each comprising binding the one or more polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the one or more polypeptides or subunits from the support material after substances not bound to the support material have been removed.
- 2. (currently amended) A Method method for detecting and/or purifying biomolecule and/or protein complexes, the method comprising the steps:
- (a) providing an expression environment containing one or more heterologous nucleic acids encoding at least two subunits of a biomolecule complex, each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,
- (b) maintaining the expression environment under conditions that facilitate expression of the one two or more subunits in a native form as fusion proteins with the affinity tags, and under conditions that allow the formation of a complex between the one two or more subunits and possibly other components capable of complexing with the one two or more subunits,
- (c) detecting and/or purifying the complex by a combination of at least two different affinity purification steps each comprising binding the one two or more subunits via one affinity tag

to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed.

- 3. (currently amended) A Method method according to claim 1 or 2, wherein between the one or more polypeptides or subunits and one or more of the affinity tags a specific proteolytic cleavage site is present in one or more of the fusion proteins which facilitates the removal of one or more of the affinity tags.
- 4. (currently amended) A Method method according to claim 3, wherein the specific proteolytic cleavage site is an enzymatic cleavage site.
- 5. (currently amended) A Method method according to claim 4, wherein the specific proteolytic cleavage site is the cleavage site for Tobacco Etch Virus protease NIA.
- 6. (currently amended) The Method method according to claim 3 5, wherein the one or more of the proteolytic cleavage site is used to cleave the polypeptide or one or more of the subunits in step (c) from the IgG binding domain of Staphylococcus protein A bound to the support material.
- 7. (currently amended) The Method method according to claim 6, wherein the affinity purification of step (c) comprises:
- (i) binding the one or more polypeptides or subunits via the one or more IgG binding domains of Staphylococcus to a support material capable of specifically binding the latter, removing substances not bound to the support material and separating the one or more polypeptides or subunits from the support material by cleaving off the IgG binding domains via the specific proteolytic cleavage site, and
- (ii) binding the polypeptides or subunit via another affintive tag to a second support material capable of specifically binding the latter, removing substances not bound to the support material and separating the polypeptide or subunit from the support material.
- 8. (currently amended) The Method method according to claim 7, wherein step (ii) is carried out before step (i).

- 9. (currently amended) The Method method according to claim 3 8, wherein the fusion protein contains a second specific proteolytic cleavage site for the removal of one or more of the other affinity tags.
- 10. (currently amended) The Method method according to claim 1 or 2 9, wherein one of the affinity tags consists of at least one calmodulin binding peptide.
- 11. (currently amended) The Method method according to claim 10, wherein a chemical agent is used to separate the one or more polypeptides or subunits from the support material.

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